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(54) Title: PROCESS FOR PREPARING A LIGNOCELLULOSE-BASED PRODUCT, AND PRODUCT OBTAINABLE BY THE PROCESS (57) Abstract A process for the manufacture of a lignocellulose-based product from a lignocellulosic material comprises treating the lignocellulosic material and a phenolic polysaccharide with an enzyme capable of catalyzing the oxidation of phenolic groups, in the presence of an oxidizing agent. Suitable phenolic polysaccharides include phenolic starches or phenolic cationic starches derived from starches obtained from sources such as potato, corn, waxy corn, wheat, rice, sorghum, waxy sorghum, sago, arrowroot or tapioca.		

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PROCESS FOR PREPARING A LIGNOCELLULOSE-BASED PRODUCT, AND PRODUCT OBTAINABLE BY THE PROCESS

FIELD OF THE INVENTION

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The present invention provides a process for producing a lignocellulose-based product, e.g. paper, paperboard (such as cardboard and linerboard), corrugated board and the like, from an appropriate lignocellulosic starting material, such as vegetable
10 fibres (e.g. vegetable fibres originating from wood, flax, cotton, hemp, jute, bagasse, and the like). The use of the process of the invention confers excellent strength on lignocellulose-based products prepared thereby.

15

BACKGROUND AND BRIEF DESCRIPTION OF THE INVENTION

Lignocellulose-based products prepared from lignocellulosic starting materials, including products manufactured starting from vegetable fibre (e.g. wood fibre) prepared by mechanical (e.g.
20 thermomechanical) pulping procedures, mechanical/-chemical pulping procedures (the latter often being denoted "semi-chemical" procedures) or chemical pulping procedures (such as kraft, sulfite or soda pulping), are indispensable everyday materials. Some of the most familiar types of such products include paper for writing
25 or printing, cardboard and corrugated cardboard.

Virtually all grades of paper, cardboard and the like are produced from aqueous pulp slurry. Typically, the pulp is suspended in water, mixed with various additives and then passed to equipment
30 in which the paper, cardboard etc. is formed, pressed and dried. Irrespective of whether mechanically produced pulp (hereafter denoted "mechanical pulp"), semi-chemically produced pulp (hereafter denoted "semi-chemical pulp"), unbleached chemical pulp or pulp made from recycled fibres (i.e. pulp prepared from
35 recycled paper, rags and the like) is employed, it is often necessary to add various strengthening agents to the pulp in order to obtain an end product having adequate strength properties. In the case of paper and board for use in packaging and the like, the

tensile strength and tear strength under dry and wet conditions are of primary importance; moreover, notably in the case of certain grades of cardboard (e.g. so-called unbleached board for the manufacture of corrugated cardboard boxes for packaging, transport and the like), the compression strength of the material is often also an important factor.

In the field of lignocellulose-based products, considerable effort has been devoted in recent years to the development and application of strengthening/binding agents or systems which are more acceptable from an environmental and toxicity point of view than those "traditionally" used. Relevant patent literature in this respect includes the following:

EP 0 433 258 A1 discloses a procedure for the production of mechanical pulp from a fibrous product using a chemical and/or enzymatic treatment in which a "binding agent" is linked with the lignin in the fibrous product via the formation of radicals on the lignin part of the fibrous product. This document mentions "hydrocarbonates", such as cationic starch, and/or proteins as examples of suitable binding agents. As examples of suitable enzymes are mentioned laccase, lignin peroxidase and manganese peroxidase, and as examples of suitable chemical agents are mentioned hydrogen peroxide with ferro ions, chlorine dioxide, ozone, and mixtures thereof.

EP 0 565 109 A1 discloses a method for achieving binding of mechanically produced wood fragments via activation of the lignin in the middle lamella of the wood cells by incubation with phenol-oxidizing enzymes. The use of a separate binder is thus avoided by this method.

US 4,432,921 describes a process for producing a binder for wood products from a phenolic compound having phenolic groups, and the process in question involves treating the phenolic compound with enzymes to activate and oxidatively polymerize the phenolic compound, thereby converting it into the binder. The only phenolic compounds which are specifically mentioned in this document, or

employed in the working examples given therein, are lignin sulfonates, and a main purpose of the invention described in US 4,432,921 is the economic exploitation of so-called "sulfite spent liquor", which is a liquid waste product produced in large quantities through the operation of the sulfite process for the production of chemical pulp, and which contains lignin sulfonates.

With respect to the use of lignin sulfonates - in particular in the form of sulfite spent liquor - as phenolic polymers in systems or processes for strengthening/binding wood products, the following comments are appropriate:

(i) lignin sulfonates available on a commercial scale are generally very impure and of very variable quality [see J.L. Philippou, Journal of Wood Chemistry and Technology 1(2) (1981) 199-227];

(ii) the very dark colour of spent sulfite liquor renders it unsuited as a source of lignin sulfonates for the production of, e.g., paper products (such as packaging paper, linerboard or unbleached board for cardboard boxes and the like) having acceptable colour properties.

The present inventors have surprisingly found that strengthened lignocellulose-based products (e.g. paper and paperboard) can be manufactured by a procedure involving the use of a combination of a polysaccharide which is substituted with at least substituents containing a phenolic hydroxy group (in the following often simply denoted a "phenolic polysaccharide"), an oxidizing agent and an enzyme capable of catalyzing the oxidation of phenolic groups by the oxidizing agent, and that products produced in this manner exhibit strength properties at least comparable to, and often significantly better than, those achievable using previously known processes.

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It is appropriate to mention here that PCT application No. PCT/DK95/00318 (unpublished at the priority date of the present application; subsequently published as WO 96/03546) discloses a

process for the manufacture of a lignocellulose-based product from a lignocellulosic material, the process comprising treating the lignocellulosic material and a phenolic polysaccharide with an enzyme capable of catalyzing the oxidation of phenolic groups, in the presence of an oxidizing agent.

It is stated in PCT/DK95/00318 that phenolic substituents in phenolic polysaccharides suited for use in the context of the invention described therein may suitably be linked to the polysaccharide species by ester linkages or ether linkages.

Types of phenolic polysaccharides mentioned in PCT/DK95/00318 include those in which the phenolic substituent of the phenolic polysaccharide is a substituent derived from a phenolic compound which occurs in one of the following plant-biosynthetic pathways: from p-coumaric acid to p-coumaryl alcohol, from p-coumaric acid to coniferyl alcohol and from p-coumaric acid to sinapyl alcohol; p-coumaric acid itself and the three mentioned "end products" of the latter three biosynthetic pathways are also mentioned in this respect. Disclosed examples of relevant "intermediate" compounds formed in these biosynthetic pathways are caffeic acid, ferulic acid (i.e. 4-hydroxy-3-methoxycinnamic acid), 5-hydroxy-ferulic acid and sinapic acid.

More specifically, PCT/DK95/00318 discloses the following types of phenolic polysaccharides as being suitable in the context of the invention described therein:

(a) phenolic arabinoxylans, phenolic heteroxylans and phenolic pectins [such as arabinoxylans and pectins containing "ferulyl" (i.e. 4-hydroxy-3-methoxycinnamyl) substituents attached via ester linkages]; and

(b) certain phenolic starches (more specifically starches which have been chemically modified by the introduction of acyl-type substituents derived from hydroxy-substituted benzoic acids, such as 2-, 3- or 4-hydroxybenzoic acid).

DETAILED DESCRIPTION OF THE INVENTION

The present invention thus provides a process for the manufacture
5 of a lignocellulose-based product from a lignocellulosic material,
the process comprising treating (i) said lignocellulosic material
and (ii) a phenolic polysaccharide other than those specifically
disclosed in PCT/DK95/00318 (*vide supra*)

10 with (iii) an enzyme capable of catalyzing the oxidation of
phenolic groups, in the presence of (iv) an oxidizing agent (more
specifically an oxidizing agent appropriate for use with the
enzyme in question, in general an oxidizing agent which in
conjunction with the enzyme is capable of bringing about oxidation
of phenolic groups).

15

Enzymes of the type(s) employed in the process of the present
invention, i.e. enzymes capable of catalyzing the oxidation of
phenolic groups, are believed to lead to the formation, in the
presence of an appropriate oxidizing agent, of radicals in the
20 aromatic moieties of phenolic substituents, such as the phenolic
functionalities in phenolic polysaccharides and in the lignin part
of a lignocellulosic substrate.

In this connection, but without being limited to any specific
25 theory, it is believed that a reaction of central importance in
the process of the invention is a reaction between phenolic
substituents (especially those on the lignocellulosic material and
the phenolic polysaccharide, respectively) which have been
"activated" by radical formation as described above.

30

With reference to the above, the order of mixing/contacting the
four components, i.e. the lignocellulosic material, the phenolic
polysaccharide, the enzyme and the oxidizing agent, is not
critical as long as the process set-up ensures that the
35 "activated" lignocellulosic material and the "activated" phenolic
polysaccharide are brought together in a way that enables them to
react in the desired manner. Thus, for example, the enzyme and the
oxidizing agent may be mixed with, or otherwise brought into

contact with, the lignocellulosic material before or after being mixed with the phenolic polysaccharide.

In the manufacture of - in particular - paper, a technically very satisfactory embodiment of the process of the invention involves the continuous spraying of a solution of the phenolic polysaccharide and a laccase [or another enzyme of the oxidase type which catalyzes oxidation of phenolic groups by oxygen (*vide infra*)] at ambient temperature (e.g. about 20-25°C) or a higher temperature (e.g. a temperature in the vicinity of 40°C) onto a thin layer of the moving lignocellulosic material (pulp) on the papermaking machine, in the presence of atmospheric air as oxygen source.

In some other embodiments of the process of the invention it may be appropriate to incubate a reaction medium containing the lignocellulosic material, phenolic polysaccharide and enzyme in the presence of oxidizing agent for a period of at least a few minutes. An incubation time in the range of from 1 minute to 10 hours will generally be suitable, although a period of from 1 minute to 2 hours is preferable.

As already indicated, the process of the invention is well suited to the production of a variety of types of lignocellulose-based products, e.g. various paper and paperboard products (such as cardboard, linerboard and the like).

The lignocellulosic starting material employed in the method of the invention can be in any appropriate form, e.g. in the form of vegetable fibre (such as fibres from wood, flax, cotton, hemp, bagasse, jute and the like), depending on the type of product to be manufactured.

It will normally be appropriate to employ the lignocellulosic material in question in an amount corresponding to a weight percentage of dry lignocellulosic material [dry substance (DS)] in the medium in the range of 0.1-90%.

The temperature of the reaction mixture in the process of the invention may suitably be in the range of 10-120°C, as appropriate; however, a temperature in the range of 15-90°C is generally to be preferred. As illustrated by the working examples provided herein (*vide infra*), the reactions involved in a process of the invention may take place very satisfactorily at ambient temperatures around 25°C.

10 Phenolic polysaccharides

As mentioned above, the phenolic polysaccharide employed in the process of the present invention is a phenolic polysaccharide other than those specifically disclosed in PCT/DK95/00318.

15 The phenolic substituent(s) in phenolic polysaccharides suited for use in the context of the present invention may suitably be linked to the polysaccharide species by, e.g., ester linkages or ether linkages.

20 Particularly suitable phenolic polysaccharides are those which exhibit good solubility in water, and thereby in aqueous media in the context of the invention.

It should be noted that the term "polysaccharide" in the context of the present invention refers not only polysaccharides *per se*, but also to derivatives - often synthetic derivatives - thereof, especially derivatives which exhibit greater water solubility than the "parent" polysaccharide.

30 More specifically, some preferred types of phenolic polysaccharides for use in the process of the present invention include the following:

(A) Phenolic starches other than those specifically mentioned in PCT/DK95/00381 (*vide supra*), i.e. other than those in which the phenolic substituents are acyl-type substituents derived from 2-, 3- or 4-hydroxybenzoic acid; and phenolic starch derivatives (i.e.

starch derivatives into which phenolic substituents have been introduced by chemical or enzymatic means).

5 The "parent" starch from which a phenolic starch or phenolic starch derivative employed in the context of the present invention is derived may, for example, suitably be any of the commercially available types of starch. These include starch from potato, corn (maize), waxy corn (waxy maize), wheat, rice, sorghum, waxy sorghum, sago, arrowroot and tapioca (cassava, manioc). Relevant
10 types of starch thus include both high-amylose starches (such as starch from so-called "high-amylose corn") and high-amylopectin starches (such as starch from waxy maize, waxy sorghum or glutinous rice). Potato starch is a very suitable "parent" starch in the context of the invention.

15

The starch derivative from which a phenolic starch derivative employed in the context of the present invention is derived may, for example, be a starch ester (e.g. a starch acetate) or an hydroxyalkylstarch (e.g. an hydroxyethyl- or hydroxypropylstarch).

20

Particularly interesting starch derivatives are so-called "cationic starches", such as those wherein the cationic functionality is of the quaternary ammonium type. Cationic starches of the quaternary ammonium type are themselves widely
25 used in the paper industry as so-called "wet-end additives" for improving, *inter alia*, strength and drainage, and as binders in coatings; one example of a commercially available cationic starch product of the quaternary ammonium type is Cerestar™ CC Bond, available through Cerestar Scandinavia A/S, Holte, Denmark.

30

Preliminary experiments by the present inventors indicate that a significant further improvement in the strength of paper products (paper, cardboard, linerboard and the like) is obtainable when a phenolic cationic starch is used in the preparation thereof in the
35 manner according to the present invention.

(B) Phenolic celluloses and phenolic cellulose derivatives (i.e. celluloses and cellulose derivatives into which phenolic

substituents have been introduced by chemical or enzymatic means). Some examples of relevant phenolic celluloses are celluloses into which have been introduced phenolic substituents of one or more of the types disclosed in PCT/DK95/00381 and listed above (*vide* 5 *supra*), e.g. ferulyl substituents, or 2-, 3- or 4-hydroxybenzoyl substituents.

Owing to the generally poor water-solubility of celluloses *per se* and, in many cases, of phenolic celluloses, it will normally be 10 preferable to employ phenolic cellulose derivatives which are derived from water-soluble cellulose derivatives. Thus, a cellulose derivative from which a phenolic cellulose derivative employed in the context of the present invention is derived may, for example, suitably be an hydroxyalkylcellulose (e.g. an 15 hydroxyethyl- or hydroxypropylcellulose), or a carboxymethyl-cellulose (CMC) or salt thereof (e.g. sodium salt, sometimes known as carmellose sodium).

(C) Phenolic polysaccharides derived from polysaccharides of the 20 following types: pectins of non-*chenopodiaceae* origin (notably pectins which do not naturally contain phenolic substituents, such as citrus pectin); galactomannans [such as guar gum or locust bean gum (*ceratonia*)]; arabinogalactan (e.g. from western larch timber); dextrans; acacia gum (gum arabic); xanthan gum; 25 tragacanth gum; and carrageenan.

Preferred types of phenolic substituents in phenolic polysaccharides employed in the context of the present invention include benzyloxy (i.e. phenylmethoxy) groups having an hydroxy 30 substituent in the aromatic ring. Examples hereof are 2-, 3- and 4-hydroxybenzyloxy. The aromatic ring may optionally further be substituted with one or more other substituents, e.g. one or more lower alkyl groups (such as methyl, ethyl, 1-propyl or 2-propyl), one or more lower alkoxy groups (such as methoxy, ethoxy, 1- 35 propoxy or 2-propoxy) and/or one or more further hydroxy groups. An example of a suitable alkoxy-substituted 4-hydroxybenzyloxy substituent is 3,5-dimethoxy-4-hydroxybenzyloxy (also known as

"syringyl").

4-Hydroxybenzyloxy and related substituents may be readily introduced into, for example, starches by a simple and straightforward chemical procedure (vide infra) employing relatively mild reaction conditions.

The amount of phenolic polysaccharide employed in the process of the invention will generally be in the range of 0.01-20 weight per cent (%w/w), typically 0.01-10 % w/w, based on the weight of lignocellulosic material (calculated as dry lignocellulosic material), and amounts in the range of about 0.02-6 % w/w (calculated in this manner) will often be very suitable.

15 Enzymes

In principle, any type of enzyme capable of catalyzing oxidation of phenolic groups may be employed in the process of the invention. Preferred enzymes are, however, oxidases [e.g. laccases (EC 1.10.3.2), catechol oxidases (EC 1.10.3.1) and bilirubin oxidases (EC 1.3.3.5)] and peroxidases (EC 1.11.1.7). In some cases it may be appropriate to employ two or more different enzymes in the process of the invention.

Among types of oxidases (in combination with which oxygen - e.g. atmospheric oxygen - is an excellent oxidizing agent), laccases have proved to be well suited for use in the method of the invention.

Laccases are obtainable from a variety of microbial sources, notably bacteria and fungi (including filamentous fungi and yeasts), and suitable examples of laccases include those obtainable from strains of *Aspergillus*, *Neurospora* (e.g. *N. crassa*), *Podospora*, *Botrytis*, *Collybia*, *Fomes*, *Lentinus*, *Pleurotus*, *Trametes* [some species/strains of which are known by various names and/or have previously been classified within other genera; e.g. *Trametes villosa* = *T. pinsitus* = *Polyporus pinsitis* (also known as *P. pinsitus* or *P. villosus*) = *Coriolus pinsitus*],

Polyporus, *Rhizoctonia* (e.g. *R. solani*), *Coprinus* (e.g. *C. plicatilis*), *Psatyrella*, *Myceliophthora* (e.g. *M. thermophila*), *Schytalidium*, *Phlebia* (e.g. *P. radita*; see WO 92/01046), *Coriolus* (e.g. *C. hirsutus*; see JP 2-238885), *Pyricularia* or *Rigidoporus*.

5

Preferred laccases in the context of the invention include laccase obtainable from *Trametes villosa* and laccase obtainable from *Myceliophthora thermophila*.

- 10 Peroxidase enzymes (EC 1.11.1) employed in the method of the invention are preferably peroxidases obtainable from plants (e.g. horseradish peroxidase or soy bean peroxidase) or from microorganisms, such as fungi or bacteria. In this respect, some preferred fungi include strains belonging to the subdivision
- 15 Deuteromycotina, class Hyphomycetes, e.g. *Fusarium*, *Humicola*, *Trichoderma*, *Myrothecium*, *Verticillium*, *Arthromyces*, *Caldariomyces*, *Ulocladium*, *Embellisia*, *Cladosporium* or *Dreschlera*, in particular *Fusarium oxysporum* (DSM 2672), *Humicola insolens*, *Trichoderma resii*, *Myrothecium verrucana* (IFO 6113), *Verticillium alboatrum*,
- 20 *Verticillium dahlie*, *Arthromyces ramosus* (FERM P-7754), *Caldariomyces fumago*, *Ulocladium chartarum*, *Embellisia alli* or *Dreschlera halodes*.

- Other preferred fungi include strains belonging to the subdivision
- 25 Basidiomycotina, class Basidiomycetes, e.g. *Coprinus*, *Phanerochaete*, *Coriolus* or *Trametes*, in particular *Coprinus cinereus* f. *microsporus* (IFO 8371), *Coprinus macrorhizus*, *Phanerochaete chrysosporium* (e.g. NA-12) or *Trametes versicolor* (e.g. PR4 28-A).

30

Further preferred fungi include strains belonging to the subdivision Zygomycotina, class Mycoraceae, e.g. *Rhizopus* or *Mucor*, in particular *Mucor hiemalis*.

- 35 Some preferred bacteria include strains of the order Actino-

mycetales, e.g. *Streptomyces spheroides* (ATTC 23965), *Streptomyces thermoviolaceus* (IFO 12382) or *Streptoverticillium verticillium* ssp. *verticillium*.

- 5 Other preferred bacteria include *Bacillus pumilus* (ATCC 12905), *Bacillus stearothermophilus*, *Rhodobacter sphaeroides*, *Rhodomonas palustri*, *Streptococcus lactis*, *Pseudomonas purrocinia* (ATCC 15958) or *Pseudomonas fluorescens* (NRRL B-11).
- 10 Further preferred bacteria include strains belonging to *Myxococcus*, e.g. *M. virescens*.

Other potential sources of useful particular peroxidases are listed in B. C. Saunders et al. *Peroxidase*, London 1964, pp. 41-

15 43.

- When employing oxidases, e.g. laccases, in the process of the invention, an amount (calculated as pure enzyme protein) in the range of 0.0001-30 mg of oxidase, e.g. laccase, per gram of dry
- 20 lignocellulosic material will generally be suitable. More typical amounts will be amounts in the range of 0.001-10 mg of oxidase (e.g. laccase) per gram of dry lignocellulosic material.

- As mentioned above, preferred laccases in the context of the
- 25 invention include *Trametes villosa* laccase, and when using this laccase in the process of the invention it will generally be appropriate to employ an amount in the range of 0.02-2000 laccase units (LACU), such as 0.01-1000 LACU, per gram of dry lignocellulosic material.

30

- When employing peroxidases in the process of the invention, an amount thereof in the range of 0.00001-30 mg of peroxidase (calculated as pure enzyme protein) per gram of dry lignocellulosic material will generally be suitable. More typical
- 35 amounts will be amounts in the range of 0.0001-10 mg, such as 0.001-1 mg, of peroxidase (calculated as pure enzyme protein) per gram of dry lignocellulosic material.

As mentioned above, preferred peroxidases in the context of the invention include *Coprinus* peroxidases, such as the previously mentioned *C. cinereus* peroxidase. When using, for example, the latter peroxidase in the process of the invention it will generally be appropriate to employ an amount in the range of 0.02-5000 peroxidase units (PODU), such as 0.1-2000 PODU, e.g. 0.1-1000 PODU, per gram of dry lignocellulosic material.

10 Determination of *T. villosa* laccase activity and *Coprinus* peroxidase activity: The determination of *T. villosa* laccase activity is based on the oxidation of syringaldazin to tetramethoxy azo bis-methylene quinone under aerobic conditions, and 1 LACU is the amount of enzyme which converts 1 μM of
15 syringaldazin per minute under the following conditions: 19 μM syringaldazin, 23.2 mM acetate buffer, 30°C, pH 5.5, reaction time 1 minute, shaking; the reaction is monitored spectrophotometrically at 530 nm.

20 With respect to, in particular, *Coprinus* (e.g. *C. cinereus*) peroxidase activity, 1 PODU is the amount of enzyme which catalyses the conversion of 1 μmol of hydrogen peroxide per minute under the following conditions: 0.88 mM hydrogen peroxide, 1.67 mM 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate), 0.1 M phosphate
25 buffer, pH 7.0, incubation at 30°C; the reaction is monitored photometrically at 418 nm.

Oxidizing agents

The enzyme(s) and oxidizing agent(s) used in the process of the invention should clearly be matched to one another, and it is clearly preferable that the oxidizing agent(s) in question participate(s) only in the oxidative reaction involved in the binding process, and does/do not otherwise exert any deleterious effect on the substances/materials involved in the process.

35

Oxidases, e.g. laccases, are, among other reasons, well suited in the context of the invention since they catalyze oxidation by

molecular oxygen. Thus, reactions taking place in vessels open to the atmosphere and involving an oxidase as enzyme will be able to utilize atmospheric oxygen as oxidant; it may, however, be desirable to forcibly aerate the reaction medium with air or
5 another oxygen-containing gas (e.g. oxygen-enriched air or, if appropriate, substantially pure oxygen) during the reaction to ensure an adequate supply of oxygen.

In the case of peroxidases, hydrogen peroxide is a preferred
10 peroxide in the context of the invention and is suitably employed in a concentration (in the reaction medium) in the range of 0.01-100 mM.

pH in the reaction medium

15 Depending, inter alia, on the characteristics of the enzyme(s) employed, the pH in the aqueous medium (reaction medium) in which the process of the invention takes place will be in the range of 3-10, preferably in the range 4-9.

20 The present invention also relates to a lignocellulose-based product obtained by, or obtainable by, a process according to the invention as disclosed herein.

25 EXAMPLES

The potato starch (potato flour) employed as described in the following was a standard Danish food-grade retail product manufactured from Danish potatoes and having a declared content of
30 ca. 80% of potato starch, the balance being water. The cationic starch (often abbreviated hereafter as CS) employed (Cerestar™ CC Bond) was obtained through Cerestar Scandinavia A/S, Holte, Denmark. 4-Acetoxybenzyl acetate was prepared from 4-hydroxybenzyl alcohol (Fluka, "purum") as described below. The laccase employed
35 was *Trametes villosa* laccase, produced by Novo Nordisk A/S, Bagsvaerd, Denmark. Pre-beaten, unbleached thermomechanical pulp (TMP) prepared from mixed Scandinavian softwood (spruce) was obtained from SCA AB, Sundsvall, Sweden.

Preparation of 4-acetoxybenzyl acetate (4-ABA): 4-Hydroxybenzyl alcohol (50 grams) was dissolved in pyridine (100 ml). Acetic anhydride (100 ml) was added, and the solution was kept at room temperature overnight. The reaction mixture was then evaporated to remove the bulk of volatile components (e.g. acetic anhydride, acetic acid and pyridine), and remaining traces of pyridine were removed by co-distillation with toluene. The resulting crude 4-ABA was used without further purification.

10

Example 1. Preparation of phenolic starch (PS)

A solution containing phenolic starch was prepared as follows:

A 2% w/w solution of potato starch was prepared by boiling potato starch in the appropriate amount of water for 2 hours. The pH of the solution was adjusted to 10-11 by addition of concentrated (ca. 33% w/w \approx ca. 11.5M) aqueous NaOH. A quantity of 4-acetoxybenzyl acetate corresponding to 5% w/w of the dry weight of the amount of starch employed was added in the form of a 10% w/w solution in ethyl acetate. The resulting mixture was then stirred at 60°C for 16 hours. The reaction mixture was allowed to cool to ambient temperature, and the pH thereof was then adjusted to 5.5 by addition of glacial acetic acid.

20

Example 2. Comparison of PS/laccase and CS in paper manufacture

Standard handsheets (ca. 60 g/m²) were prepared from TMP in accordance with the SCAN standard C26:76. Four dried sheets were then immersed and soaked in a freshly prepared aqueous solution (1.2% w/w; temperature 25°C) of PS (prepared as described in Example 1, above) to which laccase (157 LACU/liter) had been added immediately prior to the immersion. A second set of four dried sheets was treated in the same way except that laccase was not added to the PS solution. The sheets were removed from the respective solutions and left at ambient temperature for 5 minutes. They were then pressed (0 \rightarrow 4 bar) in a sheet press and dried at ca. 105°C using a hot-plate drier.

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For comparison purposes, a third set of four sheets was treated in a manner completely analogous to that described above for the

second set of sheets (i.e. in the absence of laccase), but using cationic starch (CS) instead of phenolic starch (PS).

A fourth set of four dried, but otherwise completely untreated, handsheets was employed as control.

The tear-strength and tensile strength of the 4 sheets in each of the four sets was measured according to SCAN P11:73 and SCAN P38:80, respectively. The weight increase due to uptake of the "modified starch" in question (PS + laccase, PS alone, or CS alone) for the first three sets of sheets was also determined. All strength and weight measurements were made after equilibrating/conditioning the sheets at 50% relative humidity and 25°C for a minimum of 12 hours.

The average values for each set of sheets are given in Table 1, below.

Table 1.

Treat- ment	Uptake (% w/w)	Tear Index ($10^{-3} \cdot \text{Nm}^2/\text{kg}$)	Tensile Index (Nm/g)	Grammage (g/m ²)	Density (kg/m ³)
Untreated	0	5.35	12.4	62.3	238.0
PS	12.5	6.83	12.2	79.6	284.2
PS/laccase	9.7	7.06	15.1	74.9	272.1
CS	5.9	6.89	15.6	65.0	248.7

The results summarized in Table 1 show that the use of phenolic starch in combination with a laccase results in enhancement of paper strength (as measured by Tear Index and Tensile Index) to an extent similar to that achieved using (non-phenolic) cationic starch.

Moreover, preliminary results indicate that the use of a combination of a phenolic cationic starch (PCS; prepared from

cationic starch of the quaternary ammonium type with chloride as counterion, using the same method as for PS) and a laccase leads to greater strength enhancement than that obtainable with CS or with PS/laccase, particularly when the laccase employed is one
5 which exhibits a low degree of sensitivity to chloride ion (such as laccase obtainable from *Myceliophthora thermophila*).

Since PS and PCS can be prepared straightforwardly and relatively cheaply from starch (*vide supra*) and cationic starch,
10 respectively, and since the use of laccases at the levels required in the process of the invention is relatively inexpensive, strength enhancement using embodiments of the process according to the present invention, exemplified here, can thus provide an attractive alternative to the more "traditional" approach
15 employing cationic starch.

CLAIMS

1. A process for the manufacture of a lignocellulose-based product from a lignocellulosic material, the process comprising: treating
5 said lignocellulosic material and a phenolic polysaccharide with an enzyme capable of catalyzing the oxidation of phenolic groups, in the presence of an oxidizing agent;

with the proviso that said phenolic polysaccharide is not
10 chosen among:

(i) ferulylated arabinoxylans obtainable from wheat bran or maize bran, and ferulylated pectins obtainable from beet pulp;

15 and

(ii) starches which have been chemically modified by the introduction of 2-, 3- or 4-hydroxybenzoyl substituents.

- 20 2. The process according to claim 1, wherein said phenolic polysaccharide is a phenolic starch or phenolic cationic starch derived from a starch selected from the group consisting of potato starch, corn starch, waxy corn starch, wheat starch, rice starch, sorghum starch, waxy sorghum starch, sago starch, arrowroot starch
25 and tapioca starch.

3. The process according to claim 2, wherein said phenolic starch or phenolic cationic starch comprises a phenolic, ether-linked substituent selected from the group consisting of 2-, 3- and 4-
30 hydroxybenzyloxy, and 3,5-dimethoxy-4-hydroxybenzyloxy.

4. The process according to claim 2, wherein said phenolic starch or phenolic cationic starch comprises an ester-linked ferulyl substituent.

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5. The process according to any one of claims 1-4, wherein said lignocellulose-based product is selected from the group consisting of paper and paperboard.

6. The process according to any one of the preceding claims, wherein said lignocellulosic material comprises vegetable fibre.
- 5 7. The process according to claim 6, wherein said vegetable fibre originates from a vegetable source selected from the group consisting of wood, flax, cotton, hemp, jute and bagasse.
8. The process according to any one of the preceding claims,
10 wherein said enzyme is selected from the group consisting of oxidases and peroxidases.
9. The process according to any one of the preceding claims, wherein said enzyme is an oxidase selected from the group
15 consisting of laccases (EC 1.10.3.2), catechol oxidases (EC 1.10.3.1) and bilirubin oxidases (EC 1.3.3.5), and said oxidizing agent is oxygen.
10. The process according to any one of the preceding claims,
20 wherein said enzyme is a laccase obtainable from a fungus selected from the group consisting of: *Botrytis* species; *Myceliophthora* species, including *Myceliophthora thermophila*; and *Trametes* species, including *Trametes versicolor* and *Trametes villosa*.
- 25 11. The process according to claim 9 or 10, wherein the reaction medium is aerated during the process.
12. The process according to any one of claims 1-8, wherein said enzyme is a peroxidase and said oxidizing agent is hydrogen
30 peroxide.
13. The process according to claim 9 or 10, wherein said lignocellulosic material is papermaking pulp, and wherein an aqueous medium prepared from said phenolic polysaccharide and said
35 enzyme is sprayed onto a thin layer of said pulp in a papermaking machine.

14. The process according to any one of the preceding claims, wherein the temperature of the reaction medium is in the range of 10-120°C, preferably in the range of 15-90°C.
- 5 15. The process according to any one of the preceding claims, wherein an amount of phenolic polysaccharide in the range of 0.1-10% by weight, based on dry lignocellulosic material, is employed.
16. The process according to any one of the preceding claims,
10 wherein the pH in the reaction medium is in the range of 3-10, preferably 4-9.
17. A lignocellulose-based product obtainable by the process according to any one of claims 1-16.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 96/00463

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: D21H 21/18, D21H 17/24

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: D21H, D21C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CLAIMS, JAPIO, PAPERCHEM, WPI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 9603546 A1 (NOVO NORDISK A/S), 8 February 1996 (08.02.96), page 7 - page 8, line 16, the claims --	1,5-17
A	EP 0433258 A1 (ENSO-GUTZEIT OY), 19 June 1991 (19.06.91), the claims --	1-17
A	US 4432921 A (ANNEGRET HAARS ET AL), 21 February 1984 (21.02.84), abstract, the claims --	1-17
A	WO 9323606 A1 (NOVO NORDISK A/S), 25 November 1993 (25.11.93), page 1, line 21 - page 2, line 7 -- -----	1-17

☐ Further documents are listed in the continuation of Box C.☒ See patent family annex.

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"Z" document member of the same patent family

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Information on patent family members

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